IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty Docket: OP DEN CAMP-1

In re application of:	Art Unit: 1652
Hubertus J. M. OP DEN CAMP *et al.*	Examiner: Christian L. Fronda
Serial No. 10/500,872	Washington, DC
Filed: July 7, 2004	March 31, 2007
For: TRANSFORMED EUKARYOTIC CELLS THAT DIRECTLY CONVERT XYLOSE TO XYLULOSE	Confirmation No. 1317

DECLARATION OF JAN A. M. DE BONT PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Dear Sir:

I, the undersigned, declare as follows:

1. I am the Manager of Research and Development at Nedalco B.V., the assignee of the above-identified application. Before joining Nedalco in 2006, I was a professor of biocatalyst engineering at Delft University of Technology and was also employed by the Dutch research organization TNO. Until 2000 I had been a professor of Industrial Microbiology at Wageningen University, in the Netherlands. I have worked in the field of microbiology, particularly industrial microbiology and biotechnology for 30 years. I have published over 180 papers in this field. A copy of my *Curriculum Vitae* is attached to this Declaration as Appendix 1. I consider myself to be an expert in the field of this invention.

2. I am familiar with this patent application and have read and understand the latest Office Action and the references cited by the Examiner in rejecting the pending claims. My primary purpose here is to discuss the unobviousness of the present claims in relation to the cited references. Mainly Claim 1 (though also certain dependent claims) was considered obvious based on either of two references combined with a published sequence.

(a) a patent to Guan *et al.* US Patent 5,643,758 (which I will call "Guan"); or

(b) Karlsson *et al.* (Eur J *Biochem.* 2001, *268*:6498-6507 (which I will call "Karlsson").

This rejection also relies on the deposited xylose isomerase gene sequence (Accession Q9P8C9) which has the sequence referred to in the application as SEQ ID NO:1.

3. It is my understanding that the Applicants are canceling certain claims, amending other claims and adding new claims in response to the present rejections. Appendix 2 to this Declaration lists several of the claims that the Applicants' attorneys have informed me they are pursuing. These claims either have been rejected for obviousness, or, as I've been told by Applicants' attorneys, may be considered by the Examiner to fall within this same rejection.

4. I summarize below my understanding of the rejection, relying on the Patent Office's language *verbatim* in some instances. The Examiner views the Guan and Karlsson references as being similar, although not identical to the claimed invention. According to the Examiner, the references differ from the invention because the yeast cells (or the cells of filamentous fungus *Trichoderma reesei*) in the references are **not** transformed with DNA encoding a xylose isomerase that has the amino acid sequence (SEQ ID NO:1) of the claimed invention. Neither do they describe cells transformed with a sequence that is at least 70% , 80%, 90%, or 95% identical with SEQ ID NO: 1. However, SEQ ID NO:1 is known from Accession Q9P8C9 (made available by the inventors' group). The Examiner has concluded based on the above references that the transformation of yeast cells (as described in Guan) or *Trichoderma reesei* cells (described in Karlsson) with the specific polynucleotide encoding xylose isomerase disclosed in the Q9P8C9 database record would have been obvious. The Examiner contends that a skilled scientist working in this field would have been **motivated** to do this due to a generalized desire "to express and purify the xylose isomerase taught by Accession Q9P8C9". Further, the Examiner believes that there would have been **a reasonable expectation of success** in achieving this goal because of the advanced state of recombinant DNA technology for heterologous or homologous expression of proteins.

5. I respectfully wish to differ from the Examiner's views and conclusions as expressed above with respect to **motivation.** Several dozen xylose isomerase sequences were already available at the time of the invention. I call the Examiner's attention to a scientific publication by some of the present inventors (which was in part the same description present in

this application): Harhangi, HR *et al.*, 2003, *Arch. Microbiol. 180*:134-141). Fig. 4A of this document shows a phylogenetic tree of xylose isomerases including the present SEQ ID NO:1 ("*Piromyces* sp. strain E2"). This Figure, which shows only a selection of all the available xylose isomerase genes at the time of the invention, already indicates that several dozen other xylose isomerases were available at that time. Moreover, with ongoing genomics research, new microbial and plant xylose isomerase sequences are becoming available in the public DNA databases almost "on a daily basis." To date, over 1500 (partial) sequences for xylose isomerases are available from public databases.

6. It is worth noting too that the present inventors were not motivated by any desire to purify this particular xylose isomerase (or to express it for that purpose) as the Examiner's discussion of motivation seems to indicate. Rather their purpose, as described and claimed, is to endow yeast cells with an improved ability to isomerize xylose into xylulose and, hence, to grow on xylose as sole carbon source, due to the commercial advantages this offers. In view of the large number of available xylose isomerase sequences that I noted above, I know of no specific motivation for choosing the particular xylose isomerase of this invention (SEQ ID NO:1) or homologues with at least 95% sequence identity from among the many available xylose isomerase sequences. I would ask the Examiner to show me evidence (other than what is in the present application) as to why a skilled scientist in this field would have been motivated to select, in particular, SEQ ID NO:1 (or its indicated close homologues) merely from its existence in a sequence database. I believe that no such evidence exists.

7. I also wish to differ from the Examiner's views and conclusions as expressed above with regard to an ***expectation of success.*** The patent application explains on page 2, line 26 to page 3, line 10, that most of the available xylose isomerase sequences for which expression in yeast had been attempted failed to produce active xylose isomerase! The only activity observed following expression in yeast was found when the xylose isomerase came from (*of all the unexpected sources*) certain thermophilic bacteria. However, at temperatures at which yeast can grow, this xylose isomerase activity was not sufficient to support growth of the cells on xylose. It is also important to appreciate that the sequence of the xylose isomerase of the invention (SEQ ID NO:1) is more similar to some of the bacterial proteins (*Bacillus*) that **failed the test** of active expression in yeast than to the enzyme from these thermophilic bacteria. From this evidence, my conclusion is that, *a priori,* the skilled scientist did not have a reasonable

expectation of success in expressing the claimed xylose isomerase in active form in a yeast or a fungus.

8. In summary, I believe that the Examiner's conclusions about obviousness did not take into account the important points I have raised above. Taking these points into consideration, it is my opinion that there would have been no motivation to make the present invention and use the indicated xylose isomerase sequences to transform yeast so that they could be advantageously grown on xylose as their carbon source, with all the economic and commercial benefit that entails. Secondly, considering the references cited and the state of the art at the time, there would not have been a reasonable expectation that the invention would succeed.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Date: March 31, 2008 /s/ Jan A.M. de Bont

Jan A.M. de Bont

Appendix 1

Curriculum Vitae of Dr. Jan A.M. de Bont

Personal

Name:	Jan A. M. de Bont
Citizenship	The Netherlands
Date of birth	March 6, 1947
Address	68 Lawickse Allee, 6707 AK Wageningen, The Netherlands
E-mail	Jan.debont@planet.nl

Employment

2006 - present	Manager R&D Royal Nedalco, Bergen op Zoom, The Netherlands
2005 - 2007	Professor of Biocatalyst Engineering (part time), Delft University of Technology.
2001 - 2006	Program Manager Biosynthesis, TNO Quality of Life, Apeldoorn, The Netherlands.
2000 - 2001	Head of Biotechnology Group at Corporate Research of Friesland Coberco Dairy Foods, Deventer, The Netherlands.
1987 - 2000	Head of the Department of Industrial Microbiology, Wageningen University, The Netherlands.
1977 - 1987	Associate Professor, Department of Microbiology, Wageningen University

Education:

Ph.D. Wageningen University, 1976
Thesis: Nitrogen-Fixing Methane-Oxidizing Bacteria
MSc Wageningen University, The Netherlands, 1972.
Education in biological aspects of water pollution control

Research Experience

In my current position at Royal Nedalco, I oversee research in the optimization of production of bioethanol from second generation feedstocks. Key elements are the optimization of (genetically-modified) yeast in alcohol production and the application of enzymes that degrade polymeric sugar compounds such as (hemi)cellulose and pectin.

At Technical University (TU) Delft, I initiated projects in the field of directed evolution of enzymes.

At TNO, my projects were directed to producing chemicals by whole cells of *Pseudomonas putida* S12. Advantage was taken of applied genomics technologies (transcriptomics and proteomics) in studying product formation by the organism. The approach typically involved the random creation of mutants that were screened via a high-throughput system for higher-producing individual cells. These mutants were subsequently grown under controlled conditions in chemostat culture and analyzed for their transcriptome and proteome, respectively. Based on the results obtained, a rational approach could be followed in further optimizing mutants by

introducing or overexpressing genes or by suppressing them. *In situ* product removal was an integral part of the work since most compounds produced are toxic to the cell.

At Friesland Coberco Dairy Foods (now FF) from 2000 - April 2001, I headed he biotechnology group being established at a newly-created corporate research facility in Deventer. My responsibilities and accomplishments included initiating a biotechnology research program, hiring new personnel, and establishing a fully operational new recombinant DNA-laboratory facility.

As head of the Department of Industrial Microbiology at the University of Wageningen (1987-2000), my responsibilities included coordinating research and teaching. This department was established in 1987 and was staffed with 5 university employees, including 2 full time associate professors. The department also had 4-6 postdoctoral fellows, 8-12 Ph.D. students, 5-10 MSc students and foreign guests.

Teaching Experience

- At Wageningen University, I taught microbial physiology, application of genetically modified microbes, and microbial production processes.
- From 1999 onwards, I co-organized (with Prof R.A. Sheldon) a one-week International Advanced Course on Biocatalysis at TU Delft.

Scientific Publications and and PhD-mentoring:

- Published 180 papers in international journals and conference proceedings (list available upon request)
- Served as mentor ("promoter") for the PhD-thesis of 20 graduate students

Additional recent professional activities

- Chairman of the program committee of the ACTS research program *Integration of Biosynthesis and Organic Synthesis* (IBOS).
- Member of the management team of the Kluyver Centre for Genomics of Industrial Fermentation
- Scientific coordinator of the section of the Economy, Ecology, Technology (EET) program of the Dutch government on sustainable production of bioethanol and lactic acid from lignocellulosic biomass.
- Member of the Board of the International Advanced Course on Biocatalysis
- Member of the management team of the B-BASIC program on white biotechnology

Selected Claims Subject to Rejection[1]

1. A cultured eukaryotic cell transformed with a nucleic acid expression construct which construct comprises:

(a) a nucleotide sequence that encodes xylose isomerase the amino acid sequence of which is at least 95% identical with SEQ ID NO:1, and

(b) operatively linked to the nucleotide sequence of (a), a promoter that drives active expression of the xylose isomerase coding sequence in the transformed cell,

wherein, said expression construct is expressible in said cell and expression thereof confers on the cell the ability to directly isomerize xylose to xylulose.

24. A transformed eukaryotic according to claim 1, wherein the nucleotide sequence encodes a xylose isomerase the amino acid sequence of which is SEQ ID NO:1.

25. The transformed eukaryotic cell according to claim 1, wherein the amino acid sequence of the encoded xylose isomerase is identical to that of a xylose isomerase that naturally occurs in a eukaryotic organism.

2. A transformed host cell according to claim 1, wherein the cell is a yeast cell.

18. The yeast cell of claim 2 that is a member of a genus selected from the group consisting of *Saccharomyces*, *Kluyveromyces*, *Candida*, *Pichia, Schizosaccharomyces*, *Hansenula*, *Kloeckera*, *Schwanniomyces*, and *Yarrowia.*

3. The yeast cell according to claim 18 that is a member of a species selected from the group consisting of *S. cerevisiae*, *S. bulderi*, *S. barnetti*, *S. exiguus*, *S. uvarum*, *S. diastaticus*, *K. lactis, K. marxianus*, and *K. fragilis*.

4. A transformed cell according to claim 1, wherein the cell is a filamentous fungus.

[1] Includes amended versions of current claims and new claims that are being added.